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Evidence of nest usurpation of *Vespa velutina* in the founding stage by *V. basalis* reveals it as a facultative social parasite (Hymenoptera, Vespidae)

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Abstract

We investigated the usurpation behavior of the hornet *Vespa basalis* Smith in nests of *V. velutina* Lepeletier. We collected 47 nests of the two species in Taiwan in their establishment and growth stages. Of these, 29 were mixed-species nests, and in at least 16 *V. velutina* was replaced by *V. basalis*. While most nests were initiated by *V. velutina*, *V. basalis* coexisted with it during the establishment stage, leading to intense conflict between the two. The multiplex PCR method gave evidence of the usurper's eggs and larvae in the mixed-species nest. Over time, the number of adults and brood in usurped *V. velutina* nests decreased, while those of *V. basalis* increased, demonstrating behavior in *V. basalis* akin to facultative social parasitism. This study provides further evidence of nest usurpation in the genus *Vespa*, along with description of complex interactions among these hornets, their natural enemies, prey, and humans in Taiwan.

Keywords

hornet, nest usurpation, social parasitism, social wasp

Introduction

In social wasps, social parasites display highly specialized nest-building and brood-care behaviors (Matsuura and Yamane 1990). Unlike free-living eusocial bees or wasps, these insects obtain resources within the host's nest through usurpation,

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which involves invading and mimicking the host (Sakagami and Fukushima 1957). This behavioral pattern is commonly observed in both intra- or interspecific interactions and is prevalent in several genera of the subfamily Vespinae: *Dolichovespula*, *Vespa*, and *Vespula* (McDonald and Matthews 1975; Martin et al. 2008; López-Osorio et al. 2015).

In the genus *Vespa*, only *V. dybowskii* André, 1884 has been identified as a social parasite. This rare species is found in eastern China, Hokkaido and the central mountainous regions of Japan, Korea, northern Myanmar, and the Ussuri region of Russia (Sakagami and Fukushima 1957; Choi et al. 2012b; Takamizawa 2005; GBIF 2025). Limited natural history observations suggest that its nest usurpation behavior involves the queen infiltrating a mature nest of either *V. crabro flavofasciata* Cameron, 1903, or *V. simillima xanthoptera* Cameron, 1903. The invading queen kills the host queen and manipulates the host workers into raising her offspring. Over time, the host workers die, leaving the nest entirely composed of the parasite's progeny, demonstrating its role as a facultative social parasite (Sakagami and Fukushima 1957).

Social parasites and their hosts are often close relatives, in line with Emery's Rule (López-Osorio 2021). This rule suggests a sister-group relationship between hosts and parasites or, at the very least, a close evolutionary association (Huang and Dornhaus 2008; Borowiec et al. 2021). However, while *V. dybowskii* and *V. crabro* together form a monophyletic group (Archer 1992), *V. dybowskii* and its more common host, *V. simillima*, do not (López-Osorio et al. 2015). More studies demonstrated that *V. crabro* and *V. simillima* workers are capable of policing behavior by detecting and removing worker-laid eggs, thereby reinforcing the queen's reproductive dominance (Foster et al. 2002; Takahashi et al. 2004, 2007). Additionally, studies on egg and cuticle chemical signals suggest that *V. dybowskii* survives, likely due to its chemically transparent eggs and the absence of reliance on host odor or egg chemical mimicry for adaptation to *V. crabro* and *V. simillima* (Martin et al. 2008).

Ross and Matthews (1991:236) mentioned unpublished observations by Matsuura, which indicated that in the early stages of nest establishment, *Vespa velutina* Lepeletier, 1836 in Taiwan could be subject to nest usurpation by *V. basalis* Smith, 1852 (Ross and Matthews 1991). Despite outlining social parasitism within the Vespinae in his major work (Chapter 6), Matsuura (1995) did not address this phenomenon for these species. Earlier studies also suggested that before the appearance of workers, the queen of *V. basalis* might replace the nest of *V. velutina* (Kuo and Yeh 1985; Yamane 1992). These studies suggest the potential for nest usurpation between *Vespa basalis* and *V. velutina*, but the specific process has yet to be confirmed.

Further observational studies on *V. basalis* have shown that during the nest construction period (Sonan 1929; Kuo and Yeh 1985), this species is believed to migrate from subterranean cavities to large trees (Kojima et al. 2011). This allows the species to establish remarkably large nests during the reproductive season, with adult populations as high as 37,000 individuals (Yamane 1992). Presumably owing to substantial harvesting pressure from humans for wasp liquor, the species has demonstrated a notable rise in aggressive behavior (Yamane 1992).

Furthermore, there is no evidence of *V. velutina* as a social parasite. Its colony cycle is annual. After mating, the queen quickly establishes a small embryo nest, usually in a closed, sheltered location, such as a wall cavity or tree hollow. In this tennis ball-sized brood nest, the queen rears her first workers (Chauzat and Martin 2009). *V. velutina* exhibits highly complex interactions with honey bees (*Apis* spp.) and the Oriental honey buzzard (*Pernis ptilorhynchus orientalis* Taczanowski, 1891) (Lu et al. 2021), and shows significant trends of expansion and invasion (Villemant et al. 2011; Choi et al. 2012a).

Interestingly, both *V. basalis* and *V. dybowskii* exhibit very distinctive coloration, with a reddish to dark brown head and thorax and a nearly black abdomen (Matsuura 1995; Archer 2012; Smith-Pardo et al. 2020). Their comparable coloration and nest usurpation behaviors suggest potential parallels in social parasitism. Given the inherent difficulties in studying these species, we present new evidence to build upon previous observations. Early-stage nests of *V. basalis* and *V. velutina* were obtained through both field collection and the help of hornet hunters. Through photographic documentation of individuals within nests and during collection, we provide clear evidence of interspecific competition between *V. basalis* and *V. velutina* during the nesting period. Due to the limitations of traditional methods in identifying brood species, we employed rapid and reliable molecular techniques (Wilai et al. 2020). Specifically, we developed species-specific primer pairs for nestmate recognition, enabling accurate discrimination of individuals involved in nest usurpation.

Methods

Nest sampling and developmental ranking

Collection of nests was over a period beginning in 2007. This was by means of both direct nest collection and nesting records from nest boxes for birds. During 2024–2025 we commissioned hornet hunters to collect early-stage hornet nests between April and June. During the early construction phase, nests and their individuals were collected using insect-catching tools, preserved fresh at low temperature, and transported back to the laboratory. Subsequently, nests of *V. basalis* and *V. velutina* were selected as research materials. The nest sizes, individuals, eggs, and larvae within the nests were recorded.

Bird nest boxes were originally designed to attract birds for nesting in the mid-altitude Guanwu region (2,000–2,500 m, 24°30'N, 121°7'E) within Shei-Pa National Park from 2010 to 2015. The boxes measured 13 × 13 × 20 cm, with a circular entrance hole of 3 cm diameter. The lid of each box was designed to open for inspection, and the boxes were installed approximately 3 meters above ground (Fig. 1A). During the birds' breeding season (March-August) the boxes were inspected weekly. Digital cameras provided images of the nest interiors.

Various species of bees and wasps utilized the boxes for nesting, including *V. velutina*, the honey bee *Apis cerana* Fabricius, 1793, and the bumble bee *Bombus flavescens* Smith, 1852 (Sung et al. 2016). During 2010 and 2015, eight nesting records of *V. velutina* queens were documented.

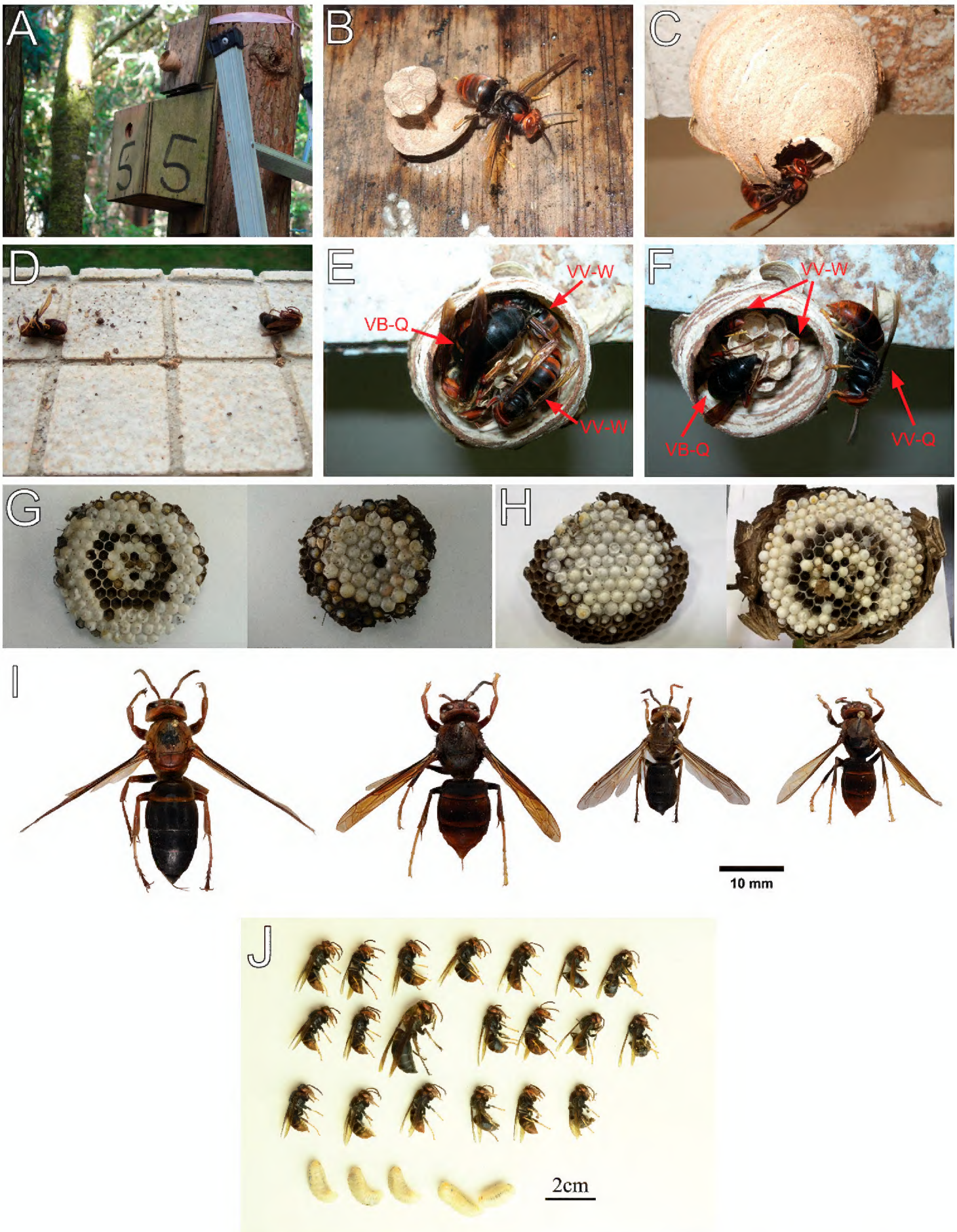


Figure 1. Details of initial and developing nests of *Vespa basalis* and *V. velutina* **A** bird nest box used as a trap; note the open lid with a top panel inside the box serving as the base for the initial nest construction **B** initial nest of *V. velutina* built on the box's top panel, showing brood cells with eggs inside **C** an embryo nest of *V. velutina* during the founding stage **D** queens of *V. basalis* (right) and *V. velutina* (left) found dead near the founding nest after fighting **E** a mixed-species nest. VB-Q: *V. basalis* queen, VV-W: *V. velutina* worker **F** a *V. basalis* queen near the entrance, with an unidentified caste of *V. velutina* standing to the right **G** developing nest of *V. velutina* showing brood cells with white cocoons **H** developing nest of *V. basalis* showing brood cells with white cocoons **I** dorsal views (left to right): *V. basalis* queen, *V. velutina* queen, *V. basalis* worker, *V. velutina* worker **J** a queen of *V. basalis* and 19 workers of *V. velutina* collected from a nest (N_12) on April 24, 2024.

Matsuura and Yamane (1990) recognized four phases of the colony cycle: colony establishment (CE), growth (CG), propagation (CP), and collapse (CC). For the collected nests, we recorded the number of combs, envelope morphology, brood (eggs, larvae, pupae), and adults (workers, males, gynes, and ovipositing queens), then classified them accordingly (Fig. 1I). Detailed classification criteria are provided in Table 1. For instance, a nest with only one queen and a single comb containing eggs and larvae would fall under the colony establishment (CE) stage.

DNA extraction and multiplex PCR experiments

For local samples, we collected four adult *V. basalis*, three adult *V. velutina*, and six *V. velutina* larvae (Table 2). DNA was extracted using the NautiaZ Tissue DNA Extraction Mini Kit (Nautia Gene, Taipei City, Taiwan) and amplified for the mitochondrial COI gene region using the universal primers LCO-1490 and HCO-2198 (Folmer et al. 1994). PCR reactions were carried out in a 25 μL volume using specific thermocycling conditions. The amplified products were analyzed via electrophoresis and subsequently sequenced by Genomics (New Taipei City, Taiwan). The sequences were compared against the NCBI database using BLAST (https://www.ncbi.nlm.nih.gov/), resulting in two mitochondrial genome sequences with 100% similarity to *V. basalis* and *V. velutina*: MK440075.1 and NC_035146.2. Specific primers were designed based on these sequences. The forward primers Basalis_F_1666_1684 5'-ATCAGGATCTTTAGGAGCC-3' and Velutina_F_2128_2146 (5'-TGGAGGTGTAGGAACAGCG-3') within the COI region, along with the universal reverse primer HCO-2198, were designed using VECTORBEE software (https://www.vectorbee.com/en/). The above sequences were aligned using MEGA 11 software (https://www.megasoftware.net/), and the specificity of the forward primers was evaluated.

Multiplex PCR was conducted in a 25 μL reaction mixture containing forward primers (0.4 μL each) and a reverse primer (0.4 μL). The thermocycling protocol consisted of an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 40 seconds, annealing at 48 °C for 90 seconds, and extension at 72 °C for 40 seconds, with a final extension at 72 °C for 10 minutes. The amplified products, approximately 657 bp for *V. basalis* and 375 bp for *V. velutina*, were verified via 1% agarose gel electrophoresis and stained with ethidium bromide.

Table 1. The colony cycle is divided into four distinct stages.

Colony stage	Nest development		Colony members						
			Immature stage			Adulthood stage			
	Comb layers	Envelope	Egg	Larva	Pupa	Worker	Male	Gyne	Queen
Establishment (CE)	1	Upper cover	+	+ / -	+ / -	-	-	-	+
Growing (CG)	> 2	Upper or partial cover	+	+	+	+	-	-	+
Propagation (CP)	Numerous	Entire cover	+ / -	+	+	+	+ / -	+ / -	+
Collapsing (CC)	Numerous	Entire cover	+ / -	+ / -	+ / -	+	+ / -	+ / -	+ / -

Table 2. Results from morphological identification of adults and molecular identification of broods in three nests. VB = *V. basalis.* VV = *V. velutina.* For details, see Fig. 5.

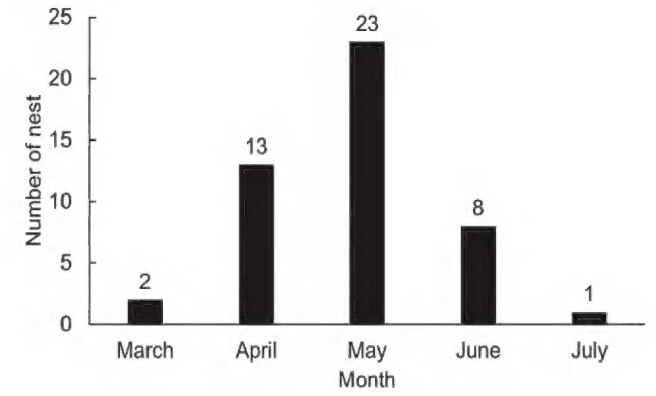
Nest code	Colony stage	Species identified by adult*	Number of workers (VV/VB)	Number of queens (VV/VB)	Number of broods (egg, larva, pupa)	PCR method	Number of broods sampled	Species identified by broods
N_4	CC	VV	1/0	1/0	32	Sanger sequencing	6	All VV
N_46	CG	VV + VB	3/22	0/1	398	Multiplex PCR	70	All VB
N_47	CG	VV + VB	10/1	0/1	290	Multiplex PCR	62	VB and VV

To evaluate the specificity of the multiplex PCR system, various sample types were tested, including single- and mixed-species samples of *V. basalis* and *V. velutina*. DNA template was serially diluted 1000-fold, from 20 to 0.02 μg/mL, and used in a multiplex PCR assay to determine the sensitivity of the primers. After confirming the successful amplification of DNA from each species, multiplex PCR testing was conducted. Finally, brood samples from two nests, N_46 and N_47, were selected for blind testing. These represent usurpation CG nests in early-stage (N_47) and late-stage (N_46) with mixed-species adults (Suppl. material 1: table S1).

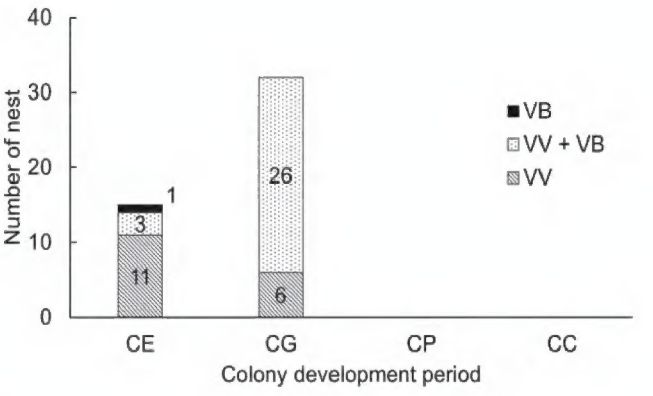
Results and discussion

Analysis based on nest development

We collected 47 early-stage nests: 9 collected by the first author, 30 via hunters, and 8 from bird nest boxes. These were collected from March to July, peaking in May with 23 nests, followed by April with 13 (Fig. 2A). We identified 15 nests in the CE stage and 32 in the CG stage; no nests in the CP or CC stage were observed in this study (Fig. 2B). Among the 9 nests collected in June and July, 4 from low-altitude areas were in the CG stage (nest code N_2, N_5 – N_6, N_46), while 5 from mid-altitude areas were in the CE stage (nest code N_36 – N_40) (Fig. 2A, B, Suppl. material 1: table S1). The nests contained 17 *V. velutina* (VV): 11 in the CE stage and 6 in the CG stage (Fig. 2B). Additionally, the hunter brought a nest containing only a *Vespa basalis* (VB) queen in the CE stage (N_17) (Fig. 2B, C). Since the frozen brood had not developed, it was identified as a single-species nest of VB. Mixed-species nests totaled 29: 3 in the CE stage and 26 in the CG stage (Fig. 2B). Monthly distribution was as follows: March had 1 CE and 1 CG nests; April had 6 CE and 7 CG nests; May recorded 3 CE and 20 CG nests; June had 4 CE and 4 CG nests; and July had 1 CE nest (Fig. 2D). Single-species nests included 17 VV and 1 VB, while there were 29 mixed-species nests (Fig. 2C). In at least 16 of the 29 mixed-species nests, VV was clearly replaced by queen of VB (Fig. 2E). Considering



A



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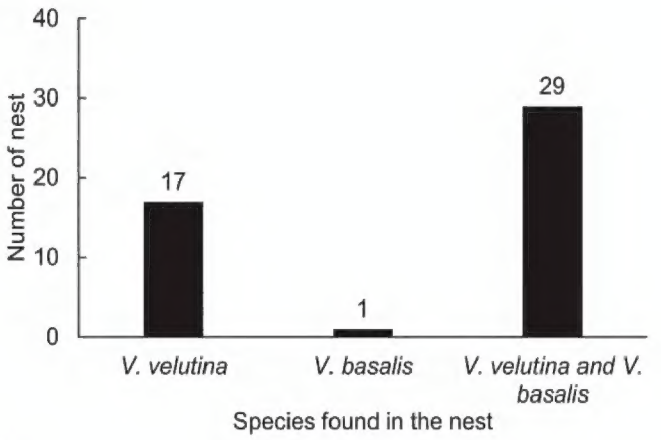

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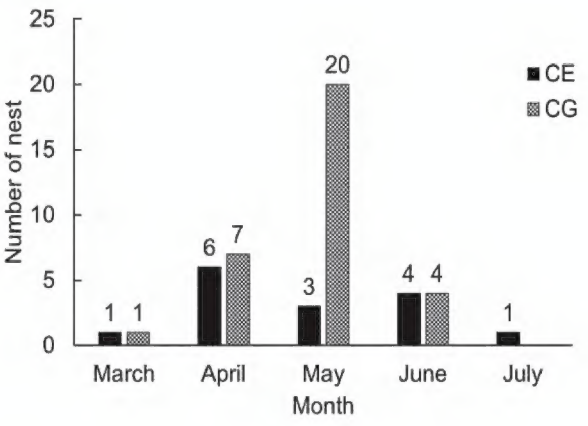

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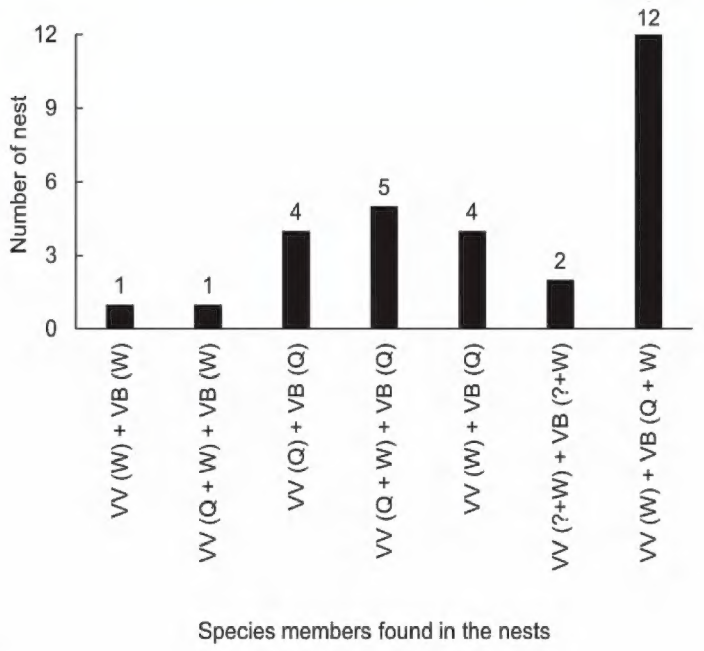

E

Figure 2. Overview of nest observations in this study **A** number of nests categorized by collection month **B** number of nests categorized by colony development stage. Stages include CE (Colony Establishment), CG (Colony growth), CP (Colony propagation), and CC (Colony collapse). VB = *V. basalis*, VV = *Vespa velutina*, VV + VB = co-occurring *V. velutina* and *V. basalis* **C** number of nests categorized by species present **D** number of nests categorized by both collection month and colony development stage **E** number of nests categorized by species composition in the nests. Q = queen present; W = worker present; ? = presence of queen undetermined.

the collection spanned multiple years, variations in climate and altitude likely influenced nest initiation as early as February or March, reaching the CG stage by April or May. In mid-altitude regions with lower average temperatures (Fig. 3), nests in the CE developmental stage were still observed in June and July.

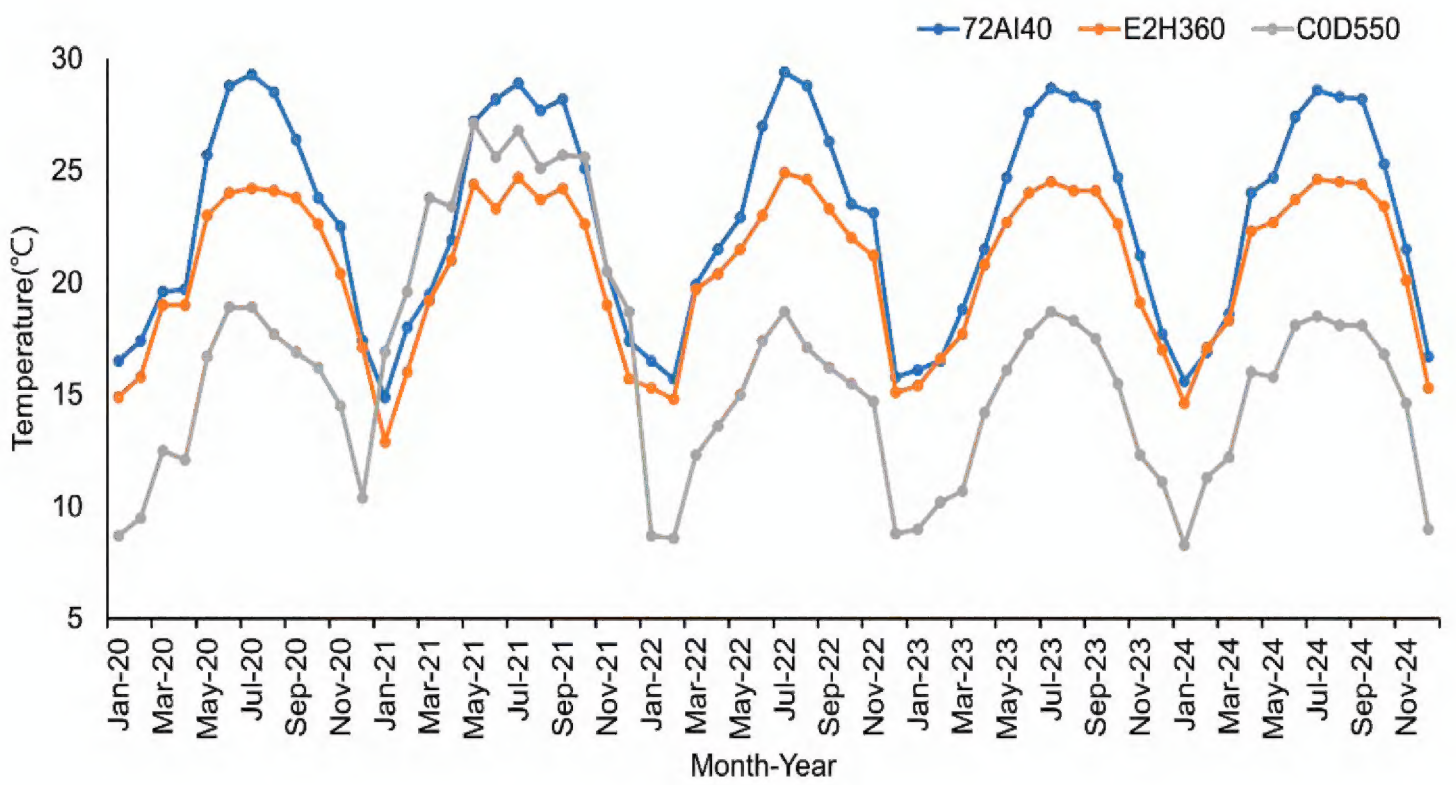

Figure 3. The average monthly temperatures (2020–2024) of the collection sites at the corresponding plain (**a**), low-altitude (**b**), and mid-altitude (**c**) stations were collected (see Suppl. material 1: table S1). Source: Central Weather Bureau (CWB) **a** 72AI40, Shu-Lin, Taoyuan District Agricultural Research and Extension Station, Ministry of Agriculture (40 m, 24°56'N, 121°23'E) **b** E2H360, Lian-Hua-Chi (681 m, 23°55'N, 120°53'E) **c** C0D550, Shei-Pa (1,956 m, 24°31'N, 121°6'E).

Visual observations of the nest-building process by the foundresses

Regarding the discovery and collection of hornet nests, we have compiled three cases to illustrate our observation records, including the establishment of the initial nest, the situation of the initial nest when the nest usurpation occurred, and the subsequent development after usurpation.

1: Observation of initial and growth of single-species nests

In the bird nest boxes at Guanwu, we recorded eight instances of initial nest construction by VV (N_35 – N_42). During the early stages, the foundress used the top panel of the boxes as a base, creating a first comb layer with several cells and an envelope (Fig. 1A, B). However, later observations showed that these colonies were abandoned and did not develop further worker populations, except one nest (N_39) had 4 VV workers until August. The reasons for abandonment remain unclear, possibly due to human disturbance or competition from other species. These observations confirm that the nests were constructed, cared for, and used for egg-laying by VV.

For nests in the growth stage, five nests (N_8, N_24 – N_25, N_32, N_34) were observed to contain VV members but no VB. The cocoons in these VV nests were

white, resembling the color of VB cocoons (Fig. 1G–H). Additionally, no nests containing only VB members were found during the collection process in the growth stage.

2: Observation of initial mixed-species nests

In late April 2007, an initial nest (N_3) was discovered at the Fushan Research Center (TFRI), and an automatic camera subsequently installed for one month. The nest was found 10 cm in diameter under a building, guarded by a VV (Fig. 1C). Nearby, dead queens of VB and VV were discovered, suggesting a battle during nest establishment (Fig. 1D). The camera also captured a Formosan macaque *Macaca cyclopis* (Swinhoe, 1862) destroying the nest. By the end of May, a smaller nest was built at the same location. Subsequent footage showed a VB approaching and entering the nest, interacting with the resident VV (Fig. 1E, F); it is unclear if a confrontation occurred. However, in the three nests (N_43 – N_45) collected at the medium altitude of Biluxi Station and Fushan Research Center (TFRI), both foundresses of VB and VV were found to coexist in some cases (Suppl. material 1: table S1), and the later emerging first batch were all workers of VV.

3: Observation of growing stage of mixed-species nests

In mid-May 2012, a growing nest was discovered on a *Sabal* sp. palm tree at the Taipei Botanical Garden (TFRI) (N_1). Upon its removal, 5 VB and 30 VV apparent workers were captured. The comb consisted of three layers; the first and second layers contained pupae, many with small holes in their caps, possibly caused by biting. Over 30 adult VB later emerged.

Additionally, in early June 2012, a small nest less than 10 cm in diameter was discovered emerging from the ground at the Fushan Research Center (TFRI) (N_2). A total of 8 VB and 4 VV workers were collected. The comb consisted of two layers: the second layer had 12 empty cells, while the first layer contained 29 capped pupal cells and 30 large larvae. Approximately 80 cells were empty and extensively covered with green mold, which was also present along the upper and peripheral sections of the comb. The nest, with about 150 cells, had no eggs or young larvae, indicating that its nest had been abandoned for some time. This abandonment could have been caused by the queen's death. Despite this, the pupae continued to emerge and develop. Notably, the coexistence of VB and VV was observed within the nest.

Notably, the VB nests that had transitioned to the social stage still included residual VV workers, as seen in 11 example nests (Fig. 1J). We found a unique case in N_15, where the queen of VV was apparently replaced. However, three queens of VB were also collected from this nest at the same time (Suppl. material 1: table S1).

DNA identification of immature individuals

The designed primer pair amplified an approximately 657 bp fragment in VB and a 375 bp fragment in VV. In single-species tests, the species-specific primers produced

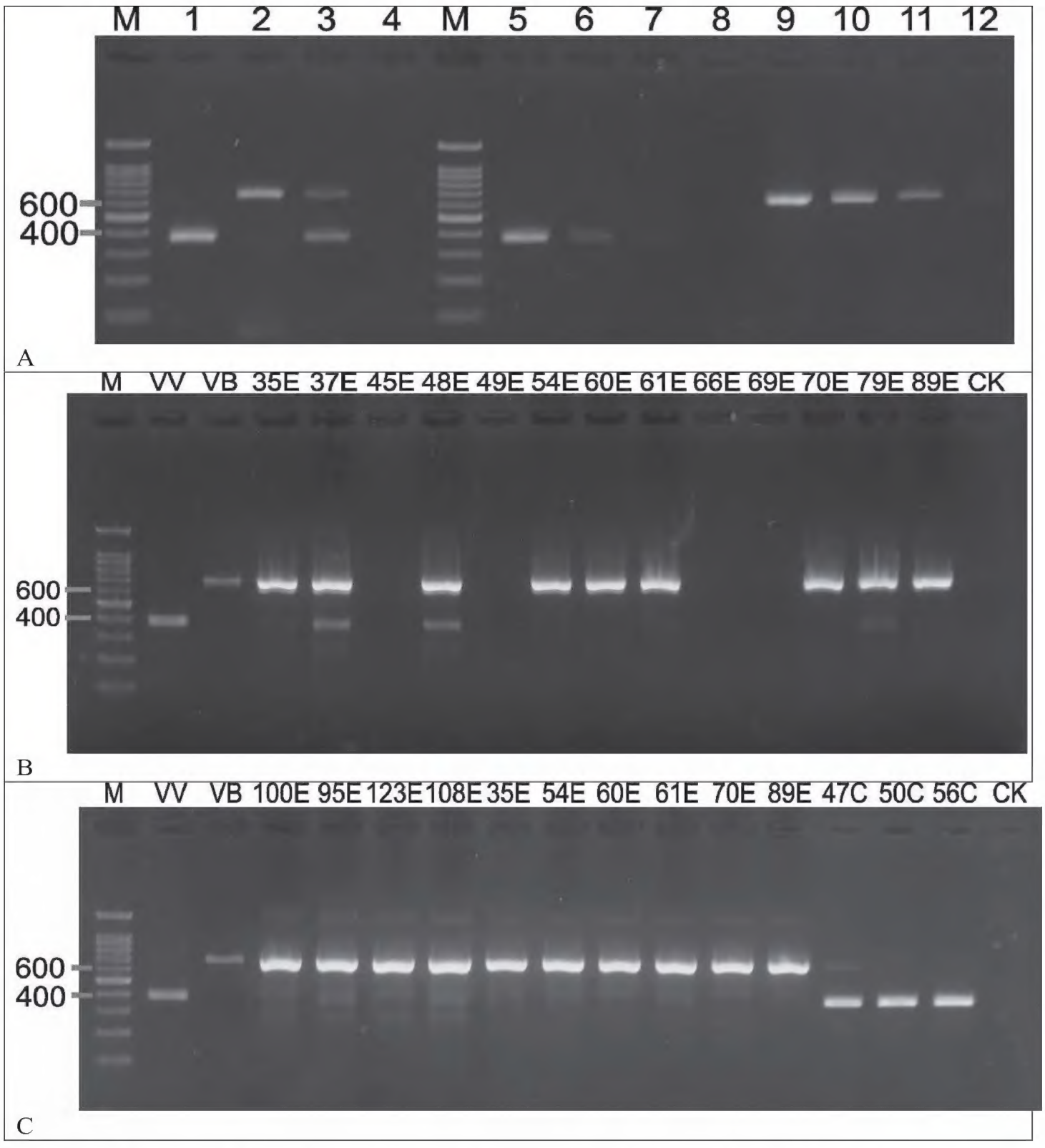

Figure 4. Diagnostic and multiplex PCR using species-specific primers **A** multiplex PCR assay. Specificity test: Lane M: DNA marker; Lane 1: *V. velutina*; Lane 2: *V. basalis* (100-fold diluted DNA); Lane 3: mixed species; Lane 4: negative control. Sensitivity test: Lanes 5–8: *V. velutina* (template concentrations: 20–0.02 μg/mL); Lanes 9–12: *V. basalis* (same gradient) **B, C** blind tests. Lane M: DNA marker; Lane VV: *V. velutina*; Lane VB: *V. basalis*; Lane CK: negative control. Samples showing a single clear band (e.g., lanes 60E, 70E, 89E and 56C) are considered normal result. In contrast, samples with no bands, multiple bands, or smearing (e.g., lanes 45E, 37E and 48E) are classified as abnormal results.

clear and distinct bands at the expected positions for their respective target species (Fig. 4A). In contrast, mixed-species DNA samples yielded two separate bands corresponding to the two species (Fig. 4A). Sensitivity assays further indicated that extracted VB DNA was typically of relatively high concentration. Optimal amplification

was achieved at approximately a 100-fold dilution, indicating that lower DNA input enhances PCR performance under these conditions (Fig. 4A).

In blind tests, the use of undiluted, high-concentration DNA may result in multiple non-specific bands or smearing, potentially complicating interpretation. In practical applications, cross-contamination may occur when both species are present in the same colony (Fig. 4B, C). For example, accidental mixing of hemolymph from larvae or eggs during sampling can lead to DNA contamination. Therefore, such ambiguous results should be excluded from downstream analyses.

Using multiplex PCR, blind brood samples were collected from two nests, N_46 and N_47 (Table 2). In nest N_46, the sample included 20 eggs, 20 larvae, and 30 pupae, all of which were identified as VB in the blind test. This result indicates that in the late stage of usurpation the colony had been completely taken over by VB.

In Nest N_47, samples were collected from a single layer of the upper comb. Particular attention was given to the central area of the comb, where uncapped cells contained eggs (e.g., samples 70E, 104E, and 61E) (Fig. 5). These eggs were likely laid after the first batch of workers had emerged from the cells, suggesting recent reuse of these cells by a queen. Additionally, many uncapped cells around the periphery of the capped area also contained eggs, indicating that these cells were recently constructed during nest expansion. Based on this, it can be inferred that the larvae or pupae in the capped cells developed earlier, and the offspring within these cells may have been produced by different queens before and after the nest was usurped.

A total of 70 samples were analyzed using both visual inspection (n = 8) and multiplex PCR (n = 62) (Table 2). After excluding ambiguous or inconclusive results, 43 samples yielded reliable species identifications. Of these, 18 eggs were identified as VB, while the more developed larvae and pupae were predominantly identified as VV (Fig. 5). These blind test results support the conclusion that all newly laid eggs in this nest belonged to VB (n = 18), with only 8 VB individuals detected among the larvae and pupae. In contrast, VV was found in 24 pupae or individuals close to eclosion, with only one larva attributed to this species. This provides strong evidence that, following the usurpation of colony N_47, the usurping *V. basalis* dominated the colony's reproductive output.

Diagnosis of nest usurpation behavior on *Vespa basalis*

Previous observations indicated that *V. basalis* and *V. velutina* queens often build nests in underground cavities, and *V. basalis* queens may replace *V. velutina* nests before the emergence of workers (Sonan 1929; Kuo and Yeh 1985). Additionally, Ross and Matthews (1991:242) cited Matsuura's unpublished data that *V. velutina* exhibits a habit of nest relocation. This study addresses the early nest establishment of these wasps and explores the less-understood phenomenon of nest usurpation.

Given the high value of nests to both host and social parasites, individuals can strategize based on their own resource-holding potential and that of their competitors to minimize the costs of competition (Green and Field 2011). Preliminary findings reveal that queens of *V. basalis* and *V. velutina* coexist during the early nest-founding stage, with

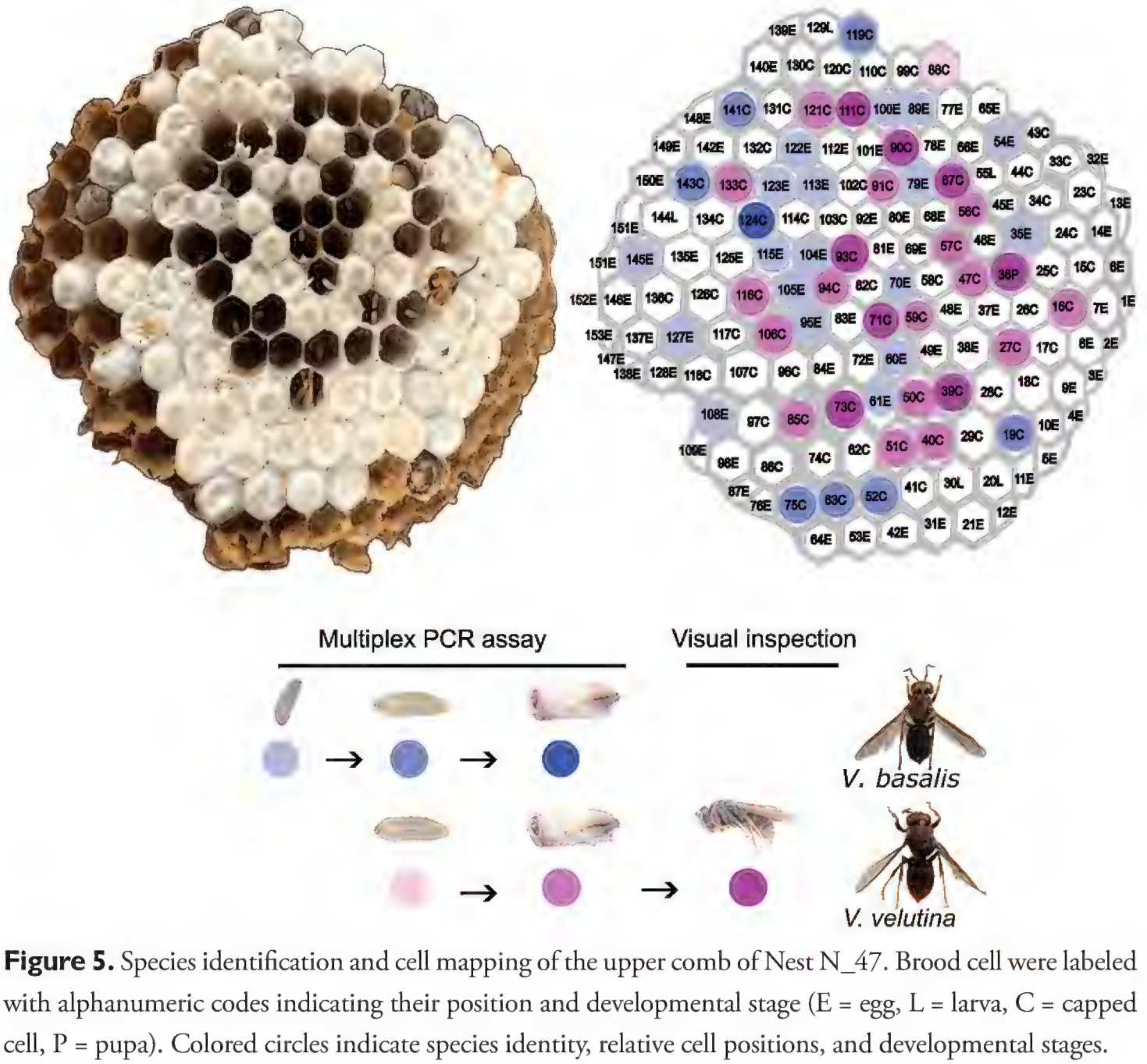

Figure 5. Species identification and cell mapping of the upper comb of Nest N_47. Brood cell were labeled with alphanumeric codes indicating their position and developmental stage (E = egg, L = larva, C = capped cell, P = pupa). Colored circles indicate species identity, relative cell positions, and developmental stages.

embryonic nests often hosting both species. The discovery of their corpses outside the nest suggests intense conflict. Based on colony structure, composition, the presence of queens, and changes in queen and worker numbers, we confirmed that most initial nests were constructed by *V. velutina* but often subsequently usurped by *V. basalis*.

Although the two species can coexist during the early stages of nest establishment, we hypothesize that *V. basalis* increases its reproductive success through usurpation. As shown in the results, this process poses significant risks to both species. The injury or death of the queen leads to losses for one or both sides, ultimately causing the failure of the entire colony. After usurping the nest, the number of *V. velutina* gradually decreased, while the number of *V. basalis* increased over time. These results suggest that usurpation behavior of *V. basalis* is similar to that observed in closely related *Vespula* and *Vespa* species showing facultative social parasitism (e.g., Sakagami and Fukushima 1957; Borowski et al. 2021).

V. velutina has demonstrated strong colonization and invasive capability in recent years (Kim et al. 2006; Choi et al. 2012a; Arca et al. 2015; Takeuchi et al. 2017).

However, studies on the natural history of this species in its native range remain limited. In Taiwan, *V. velutina* is a common species, often outnumbering other hornet species in both distribution and collection frequency (Starr 1992; Chao 2012). Compared to other native or invasive hornets, its threat to honey bee colonies in apiaries is moderate (Chao et al. 2002; Lu et al. 2021).

The development of a multiplex PCR system enables accurate identification of brood species in large quantities. Compared to traditional sequencing, this approach significantly reduces effort and cost (Sinaie et al. 2018). In addition, it allows us to determine which queen produced which offspring within a nest and clearly reveals the temporal sequence of reproduction by the two species in mixed-species colonies. Our analysis indicates that a usurping *V. basalis* queen is capable of taking over a developing nest and producing her own offspring. These offspring eventually became dominant, replacing the original *V. velutina* colony. The multiplex PCR system provides accurate, low-cost, and rapid species identification, and may offer valuable insights for future research, for example on the relationship between *V. basalis* and *V. velutina* broods within the same nest, the chemical profiles of the brood, or the behavioral mechanisms underlying brood recognition by adult wasps.

In contrast, *V. basalis* is mainly distributed in mid-elevation areas (Starr 1992). Due to its high aggression toward animals and the economic value of hornet liquor production, previous studies have suggested that this species faces significant pressure from human harvesting (Yamane 1992). During its developmental stages on trees, both *V. basalis* and *V. velutina* are frequently preyed upon by the Oriental honey buzzard (*P. ptilorhynchus orientalis*) (RRGT, 2011). It is noteworthy that *V. basalis* may be influenced by intrinsic factors and exhibits a form of facultative social parasitism akin to that of *V. dybowskii*. Our observations suggest that complex interactions among external factors exist between mammals, hornets, honey bees, birds, and humans.

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Supplementary material 1

Records of hornet nests *Vespa basalis* and *V. velutina* collected in this study

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Data type: pdf

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